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Terbutryn resistance in a purple bacterium can induce sensitivity toward the plant herbicide DCMU

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Chromatophores of the mutant T4 (TyrL222→Phe) from *Rhodopseudomonas viridis*, resistant toward the triazine herbicide terbutryn (2-methylthio-4-ethylamino-6-tertbutylamino-s-triazine), were studied by EPR and optical spectroscopy. IC_{50} values were measured with electron transfer inhibitors of the triazine, triazinone, phenol and urea classes. The mutant T4 is the first purple bacterium known so far, which is highly sensitive to the plant herbicide DCMU (3-(3,4-dichlorphenyl)-1,1-dimethyl-urea, also known as diuron).

Herbicide-resistant mutant; Reaction center, Photosynthetic; Purple bacteria; Diuron; Terbutryn

1. INTRODUCTION

Herbicides of the triazine class block electron transfer to the secondary quinone, Q_B, in photosynthetic reaction centers (RCs) of purple bacteria, cyanobacteria and higher plants [1–3]. However, despite significant sequence homologies between the Q_B binding site of purple bacteria and PS II, herbicides of the urea and phenol types are ineffective in purple bacteria ([4] see also [5]). All photosystem II RCs bind to the same site of the protein, but at different overlapping domains [5]. For terbutryn, a triazine herbicide, the site of binding to the RC of *Rhodopseudomonas (Rps.) viridis* was established by X-ray crystallography [6]. From the structure it appears that a hydrogen bond is possible between the ethylamino nitrogen of terbutryn and the side chain oxygen of serine L223. A second hydrogen bond is likely between the peptide nitrogen of isoleucine L224 and N3 of the s-triazine ring system. Valine L220, phenylalanine L216 and isoleucine L229 make extensive contacts with the herbicide. Several mutants from *Rps. viridis*, which are resistant toward terbutryn, have been isolated by selecting for photosynthetic growth in the presence of terbutryn [7,8]. Their characterization using genetic analysis, EPR and optical spectroscopy showed that some features of these mutants have not been observed in herbicide resistant mutants of other purple bacteria, higher plants or algae [8]: (i) in two mutants two amino acids were changed; (ii) the mutant T1 (SerL223⟶Ala, ArgL217⟶His) showed a higher affinity for the secondary quinone Q_B than the wild type; (iii) the mutant T4 (TyrL222⟶Phe) showed a $Q_B^-Fe^{2+}$ EPR signal having a $g = 1.93$ peak, that is different from the signals obtained for the wild type and the other mutants, but very similar to those of PS II [9,10] and *Rhodospirillum (R.) rubrum* [11]. Since tyrosine L222 forms no contacts with terbutryn, it is not obvious why the change of tyrosine L222 to phenylalanine results in herbicide resistance [8]. Tyrosine L222 seems to form a hydrogen bond with the backbone carbonyl group of aspartic acid M43 [8]. This interaction might be crucially important in stabilizing the tertiary structure of this region. The observation of a PS II-type EPR

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signal made the mutant T4 the most obvious choice for testing PS II-type herbicides.

2. MATERIALS AND METHODS

Rps. viridis DSM133 was grown as described [12]. The herbicide resistant mutant T4 was grown in the presence of 100 μM terbutryn. Chromatophores have been prepared using the standard procedure [12]. They were resuspended in 50 mM Mes buffer, pH 7.0. The concentration of bacteriochlorophyll was measured via the absorption at 1015 nm (ϵ_{1015} = 100000 [13]).

The bacteriochlorophyll concentration in the EPR measurements was 1.3 mg/ml for the wild type and 1.5 mg/ml for T4. The setup of the EPR machine, the conditions of the measurements and all sample handling were as described previously [8]. 500 μM DCMU was added from a 0.1 M stock solution in DMSO, before DAD and ascorbate.

The bacteriochlorophyll concentration in the optical measurements was 0.08 mg/ml for the wild type and T4. The biphasic recombination from the states $P^+Q_A^-$ or $P^+Q_B^-$ was recorded at 1300 nm. The conditions were exactly as described in [8], except that only four flashes were averaged. The inhibitors were added in DMSO. DCMU was from Sigma, all other inhibitors were from Riedel-de-Haen.

3. RESULTS AND DISCUSSION

Fig.1a shows the EPR spectrum generated in *Rps. viridis* wild-type chromatophores when given a single flash at room temperature. The signal is typical of $Q_B^{\cdot-}Fe^{2+}$ in this species [14] having a marked double peak at g = 1.84 and a narrow linewidth (~185 G, peak to trough) compared to that of $Q_A^{\cdot-}Fe^{2+}$ (~330 G). The presence of DCMU had no effect on the EPR spectrum (fig.1b). The $Q_B^{\cdot-}Fe^{2+}$ signal generated by a flash in the T4 mutant (TyrL222⟶Phe) is quite different from the wild-type (fig.1c). As described earlier [8], it has a smaller peak at g = 1.84 and exhibits a major feature at g = 1.93. When DCMU was present in the chromatophores of T4, a single flash generated an EPR signal which has a quite different shape (fig.1d). This signal is attributable to $Q_A^{\cdot-}Fe^{2+}$ since it is almost identical to the $Q_A^{\cdot-}Fe^{2+}$ signal generated in T4 and Wt by continuous illumination in the presence of exogenous electron donors (not shown, but see [8]). DCMU does not significantly modify the $Q_A^{\cdot-}Fe^{2+}$ signal in T4 nor in the WT (not shown). The $Q_A^{\cdot-}Fe^{2+}$ signal in the T4 mutant is very similar to that in the wild type [8] and is not greatly influenced by DCMU (not shown). The results of fig.1 show that in the T4 mutant, electron transfer is blocked on the level of Q_A^-.

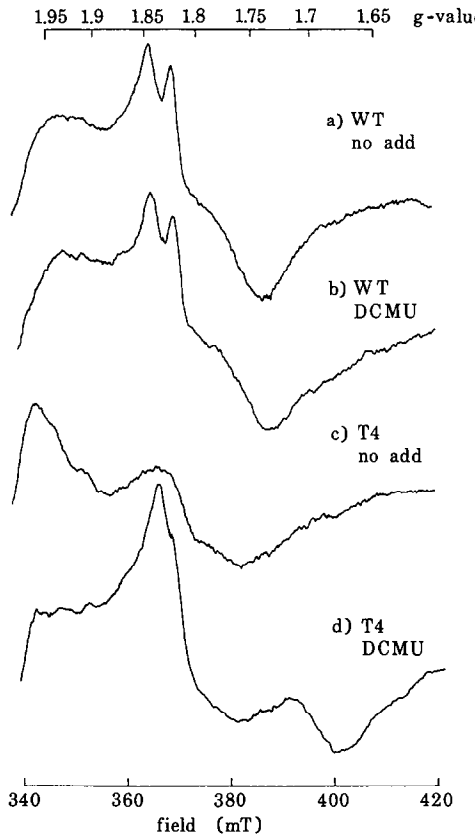

Fig.1. EPR spectra of chromatophores from *Rps. viridis* wild type and the mutant T4. All signals shown here were obtained after one flash at RT, prior to freezing in the dark; no add = without herbicide; DCMU = 500 μM. Conditions: 1.3 mg/ml (wild-type) and 1.5 mg/ml (T4) bacteriochlorophyll; Mops buffer (50 mM, pH 7.0); sample volume 250 μl; DAD 1 mM, ascorbate 5 mM; frequency 9.44 GHz; modulation amplitude 12.5 G; microwave power 8 dB; temperature 4.5 K.

Relative binding affinities were determined on chromatophores measuring the charge recombination at 1300 nm, see [8]. The signals were clearly biphasic (not shown); a fast phase ($t_{1/2}$ = 1 ms, *Rps. viridis* wild type [15]) is attributed to the recombination from $P^+Q_A^-$ and a slow phase ($t_{1/2}$ = 100 ms [15]) to the recombination from $P^+Q_B^-$ respectively [15–17]. Without additions the fraction of the slow phase was about 80% for the wild type and 40% for T4. The decreased fraction of the slow phase in chromatophores of T4 could, at least partially, reflect the lower binding affinity of Q_B in that mutant [8].

I_{50} values for eight different inhibitors have been determined from their effect on the suppression of

Table 1

Summary of the IC_{50} values (in μM) from *Rps. viridis* wild type and T4

Inhibitor	Wild type IC_{50}	T4 IC_{50}	*R/S*
Terbutryn	1.2	800	660
Atrazine	17	>10000	>600
Ametryn	4.5	1500	330
Desmetryn	20	1000	50
Metribuzin	>10000	>10000	
o-Phenanthroline	32	300	10
DCMU	>10000	8	<0.0001
Ioxynil	>10000	100	<0.01

The chromatophore concentration corresponds to 0.08 mg Bchl/ml. *R/S* gives the relative resistance (IC_{50} of T4/IC_{50} of wild type)

the slow phase and on the simultaneous increase of the fast phase (table 1). The triazinone herbicide, metribuzin, was ineffective in the wild type as well as in T4. All triazines used here blocked the electron transfer in the wild type with the following effectiveness: terbutryn > ametryn > atrazine ≥ desmetryn (see also [18]). In T4 the activity of the triazines was greatly reduced and their order of effectiveness is also modified: terbutryn > desmetryn > ametryn > atrazine. The solubility was limiting for atrazine. DCMU and ioxynil were also tested. Both were ineffective in the wild type, as expected. However, much to our surprise, T4 was highly sensitive toward DCMU and markedly sensitive toward ioxynil. At low concentrations of DCMU (<100 μM), the fraction of slow phase was decreased without changing the kinetics, at high concentrations of DCMU (>200 μM) an intermediate phase with a small amplitude became evident in T4 (not shown). The I_{50} value of 8 μM for DCMU showed that it is as effective an inhibitor in T4 as the triazines are in the wild-type. The sensitivity of T4 toward ioxinyl is less marked.

From the data presented here one can only speculate about possible changes in the three-dimensional structure of the Q_B site that might explain the sensitivity toward DCMU in T4. An X-ray structure analysis of T4 is underway to answer those questions. The difference Fourier analysis of the mutant T1 at 2.5 Å resolution showed that the changed residues are clearly visible [19]. Currently, the sensitivity toward DCMU makes the mutant T4 a particularly interesting model for the herbicide binding niche of PS II.

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REFERENCES

[1] Velthuys, B.R. (1981) FEBS Lett. 126, 277–281.
[2] Wraight, C.A. (1981) Isr. J. Chem. 21, 348–354.
[3] Kyle, D.J. (1985) Photobiochem. Photobiol. 41, 107–116.
[4] Stein, R.R., Castellvi, A., Bogacz, J.P. and Wraight, C.A. (1984) J. Cell Biochem. 24, 243–259.
[5] Trebst, A. (1987) Z. Naturforsch. 42c, 742–750.
[6] Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.
[7] Sinning, I. and Michel, H. (1987) Z. Naturforsch. 42c, 751–754.
[8] Sinning, I., Michel, H., Mathis, P. and Rutherford, A.W. (1989) Biochemistry 28, 5544–5553.
[9] Rutherford, A.W., Zimmermann, J.-L. and Mathis, P. (1984) in: Advances in Photosynthesis Research, vol.I (Sybesma, C. ed.) pp.445–448, Nijhoff, Dordrecht, The Netherlands.
[10] Zimmermann, J.-L. and Rutherford, A.W. (1986) Biochim. Biophys. Acta 851, 416–423.
[11] Beijer, C. and Rutherford, A.W. (1987) Biochim. Biophys. Acta 890, 169–178.
[12] Michel, H., Weyer, K.A., Grünberg, H., Dunger, I., Oesterhelt, D. and Lottspeich, F. (1986) EMBO J. 5, 1149–1158.
[13] Olson, J.M. and Nadler, K.D. (1965) Photochem. Photobiol. 4, 783–791.
[14] Rutherford, A.W. and Evans, M.C.W. (1979) FEBS Lett. 104, 227–230.
[15] Shopes, R.J. and Wraight, C.A. (1985) Biochim. Biophys. Acta 806, 348–356.
[16] Carithers, R.P. and Parson, W.W. (1975) Biochim. Biophys. Acta 387, 194–211.
[17] Fleischmann, D. (1978) in: The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R. eds) pp.513–521, Plenum, New York.
[18] Shopes, R.J. and Wraight, C.A. (1987) in: Progress in Photosynthesis Research, vol.II (Biggins, J. ed.) pp.397–400, Nijhoff, Dordrecht, The Netherlands.
[19] Sinning, I., Koepke, J., Schiller, B., Mathis, P., Rutherford, A.W. and Michel, H. (1989) Proc. of the 8th Intern. Congress on Photosynth., in press.